Lipid-protein interactions. The leucine transport system of Lactococcus lactis.

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SUMMARY

The branched-chain amino acid (*Bca*) transport system of the homofermentative lactic acid bacterium *Lactococcus lactis* is a secondary transport protein, which translocates specifically the amino acids L-leucine, L-isoleucine and L-valine in symport with one proton across the cytoplasmic membrane. It is an integral membrane protein from which a large part is embedded in the cytoplasmic membrane. The lipids of the cytoplasmic membrane have been isolated and analyzed, both with respect to the lipid headgroup and fatty acyl chain composition. The major lipid species of *L. lactis* subsp. *cremoris* are acidic phospholipids (phosphatidylglycerol and cardiolipin), glycolipids and glycerophosphoglycolipids (Chapter 2). The major fatty acids identified in a total lipid extract from *L. lactis* are palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$) and the cyclopropane-ring containing lactobacillic acid ($C_{19\omega}$) (Chapter 3).

The lipid requirement of the *Bca* transport system has been studied, using a model system in which isolated cytoplasmic membrane vesicles were fused with liposomes, composed of a defined lipid composition. Fusion, as accomplished by a freeze/thaw-sonication procedure, results in the formation of closed hybrid membranes, which retain the energy conserving properties. This procedure has been used to enrich the membrane vesicles with exogenous lipids, up to 95 %. Fusion appeared to be independent of the composition of the liposomes. Leucine transport activity could be driven by an artificially generated protonmotive force (Δp) or its components, an electrical potential ($\Delta\psi$, inside negative) and a pH gradient (ΔpH, inside alkaline). Leucine counterflow uptake, which is independent of the magnitude of the Δp, could be induced by applying an outwardly-directed leucine concentration gradient.

High transport activities were observed in hybrid membranes containing the natural lipid mixtures extracted from *L.lactis* or *Escherichia coli*; aminophospholipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS); or glycolipids, monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (DGDG) and the neutral glycolipid fraction isolated from *L. lactis*. In contrast, phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidic acid (PA) and cardiolipin (CL) were unable to activate the leucine transport protein. In mixtures of PC and methylated derivatives of PE, leucine uptake decreased with increasing degree of methylation of PE. Because both aminophospholipids and glycolipids can form hydrogen bonds in contrast to the other lipids, it was concluded that hydrogen-bonding plays an important role in the lipid-protein interactions of the *Bca* carrier. Aminophospholipids in Gram-negative bacteria and glycolipids in Gram-positive bacteria appear to have similar functions with respect to solute transport (Chapter 2).

To determine the influence of the fatty acyl chain composition on leucine transport activity, liposomes were prepared from equimolar mixtures of synthetic PE and PC with *cis* mono-unsaturated acyl chains, varying in length from 14 to 22 C-atoms. Maximal transport activity was obtained with a fatty acid acyl chain carbon number of 18, providing an optimal membrane thickness for the leucine transport protein. The degree of matching of the lipid molecules with the hydrophobic thickness of the carrier appeared to be a second important parameter in lipid-protein interactions

(Chapter 3).

The effect on leucine transport of the degree of unsaturation of the phospholipid acyl chains was investigated in hybrid membranes composed of equimolar mixtures of synthetic PE/PC in which the number of *cis* double bonds varied between 1 and 3. Both the accumulation level and initial rate of leucine uptake decreased with increasing number of double bonds. The reduction in transport activity could be correlated with an increase in the passive permeability of the membranes to leucine. The overall fluidity of these hybrid membranes was hardly affected. It is concluded that the degree of lipid acyl chain unsaturation has a minor direct effect on the activity of the *Bca* carrier, but it affects strongly the passive permeability of the membrane (Chapter 4).

The hybrid membrane system has also been used to investigate the effect on the activity of the *Bca* carrier of membrane-spanning lipids, extracted from the extreme thermophilic archaebacterium *Sulfolobus acidocaldarius*. These lipids exist predominantly as bipolar lipids composed of macrocyclic tetraethers with polar heads linked by two hydrophobic C_{40} phytanyl chains. Each tetraether lipid molecule spans the entire membrane, resulting in a monolayer organization. Hybrid membranes composed of mixtures of monolayer lipids and bilayer lipids (PC) revealed an increase in transport activity with increasing content of monolayer lipid. Transport activity was optimal at a one-to-one ratio of PC to *S. acidocaldarius* lipids. Stimulation of the *Bca* carrier was attributed to the hydrogen-bond forming sugar residues of the tetraether lipids. A further increase in *S. acidocaldarius* lipid content, however, resulted in decreased transport activity. This inhibitory effect is most likely caused by an increased rigidity of the membrane, due to the membrane-spanning nature of the tetraether lipids; a comparable effect of membrane fluidity on leucine transport activity of *L. lactis* subsp. *cremoris* has been observed with increasing the cholesterol content in hybrid membranes composed of soybean PE/egg yolk PC (3:1, mol/mol) [Zheng, T., Driessen, A.J.M. and Konings, W.N. (1988) *J. Bacteriol.* **170**, 3194-3198] (Chapter 5).

In a second modelsystem isolated membrane vesicles of *L. lactis* were solubilized with octylglucoside in the presence of exogenously added lipids and reconstituted into proteoliposomes. Transport activity was recovered only when solubilization was performed in the presence of acidic phospholipids. In the absence of these phospholipids the transport protein was irreversibly inactivated. Similar results have been obtained for the arginine-ornithine exchange protein from *Pseudomonas aeruginosa* and *L. lactis*. The lipid headgroup requirement for a functional reconstituted transport protein could be fulfilled by the addition of aminophospholipids or glycolipids during the reconstitution step. It is concluded that acidic phospholipids protect the transport protein against delipidation, thereby preventing protein aggregation, which would result in inactivation (Chapter 6).

In summary, it is concluded, that a functionally reconstituted leucine transport system of *L. lactis* is affected by bilayer features in the following order of importance: lipid headgroup (H^+-bonding) > acyl chain carbon number (thickness) > cholesterol (fluidity) > acyl chain unsaturation (indirect by permeability changes).